CONTRIBUTIONS TO OUR KNOWLEDGE OF THE ACTINOMYCETALES. II.

The Definition and Subdivision of the Genus Actinomyces, with a Preliminary Account of Australian Soil Actinomycetes.

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(Plates xix–xx.)

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Introduction.

Few groups of microorganisms have caused more confusion in systematic respect than the genus *Actinomyces*. This confusion exists firstly as to the nomenclature, secondly as to relation to other genera and families, and thirdly in the problem whether *Actinomyces* should be included in the bacteria at all or relegated to the *Fungi imperfecti*.

Since Cohn (1875) first described an *Actinomyces* (termed *Streptothrix* by him), more names have been given to this than to any other genus of bacteria. Breed and Conn (1919), who have revised the question of nomenclature very carefully, conclude that *Actinomyces* Harz (1877) is the only valid generic name, but it is still quite common to meet the names *Streptothrix* and *Nocardia* in medical literature. Most of the earlier authors (Almquist, 1890; Boström, 1890; Kruse, 1896) regarded these organisms as "pleomorphic bacteria", and several points of similarity between them and the tubercle and diphtheria bacilli were discovered, such as the branching growth of the latter organisms, acid-fastness, and the ability of the tubercle bacillus and related forms to produce an actinomycosis-like growth in the animal organism. Lachner-Sandoval (1898) was the first to unite *Actinomyces* with the genera *Mycobacterium* and *Corynebacterium* (Lehmann and Neumann, 1896) into a family *Actinomycetes*. This name has later been altered to *Actinomycetaceae* by Buchanan (1918), who also (1917) erected an order *Actinomycetales*. This uniting of *Actinomyces*, *Mycobacterium* and *Corynebacterium* has been adopted in most treatises on systematic bacteriology (Lehmann and Neumann, 1896-1927; Orla-Jensen, 1909; Castellani and Chalmers, 1919; Bergey, 1923-1930; Enderlein, 1925; Janke, 1929; and several others), although the nomenclature is not uniform, and opinions are divided as to whether this group of organisms should be considered an Order (Buchanan, Bergey, Lehmann and Neumann) or only a family (Enderlein, Janke).

In the most recent edition of Lehmann and Neumann's Bakteriologische Diagnostik (1927) we find the following system of classification:

Order.	Family.	Genus.
Actinomycetales	Proactinomycetaceae	*Corynebacterium*
		Mycobacterium
	Actinomycetaceae	*Actinomyces*

The family name *Mycobacteriaceae* (Chester, 1897, cit. after Buchanan, 1925) has here been replaced by *Proactinomycetaceae* L. and N.

In the various editions of Bergey's Manual of Determinative Bacteriology (1923-1930) we meet an ever-increasing tendency to make the Order *Actinomycetales* what the *Fungi imperfecti* are among the Eumycetes—a heterogeneous collection of forms which cannot conveniently be placed elsewhere in the system. In the last edition (1930) the main points in the definition of *Actinomycetales* are: "Cells usually elongated, frequently filamentous and with a decided tendency to the development of branches, in some genera giving rise to the formation of a definite branched mycelium.—Usually Gram-positive. Non-motile. . ."

The Order is represented by two families—*Actinomycetaceae* and *Mycobacteriaceae*. The former contains *Actinomyces*, *Actinobacillus*, *Leptothricia*, and *Erysipelothrix*, the latter *Mycobacterium*, *Corynebacterium*, *Mycoplana*, *Fusiformis*, *Pfeifferella*, *Cytophaga*, *Cellvibrio*, and *Cellfalcicula*. The genus *Actinobacillus* Lignières and Spitz (1904) is, as pointed out by Magnusson (1928), hardly more related to *Actinomyces* than is the coli bacillus; the evidence brought forth by Nepomnjaschy (1930) in favour of a cyclogenetical relationship between *Actinobacillus* and *Actinomyces* is not entirely convincing. The genera *Leptothricia* and *Erysipelothrix* resemble *Actinomyces* only in the formation of filaments under certain conditions, which is true of many other bacteria, and the former genus lacks the property of branching which should characterize the Order Actinomycetales. As to the genera of Mycobacteriaceae, *Mycoplana* Gray and Thornton (1928) has been very little studied; it may possibly be closely related to *Mycobacterium*, as a motile parallel to this, but in that case the definition of Actinomycetales should not include non-motility as a constant character. The position of *Pfeifferella*, the organism of glanders, is doubtful; it is generally considered closely related to the corynebacteria, although Ørskov (1923) expresses grave doubt as to this. *Fusiformis* seems, as shown by Sanarelli (1927), to be related to the spirochetes rather than to the actinomycetales. Finally, as to the genera *Cytophaga*, *Cellvibrio*, and *Cellfalcicula*, it is hardly possible to see any valid reason for including them in the actinomycetales. *Cellvibrio* Winogradsky (1929) seems to differ from the familiar genus *Vibrio* only in its power of decomposing cellulose, and it may well be questioned whether this is a valid reason for making it a separate genus (*cf.* Kalninš, 1930). In the same way *Cellfalcicula* Winogradsky (1929) seems quite indistinguishable from *Cellulomonas* McBeth and Scales, a genus which Bergey places under the Order Eubacteriales; of this genus, Skinner (1929) has aptly pointed out that the power of decomposing cellulose is no valid reason for establishing it as a genus apart from *Bacterium*. The genus *Cytophaga* Winogradsky (1929) also lacks every point of resemblance to the actinomycetales. Its "sporoids" are, as pointed out by Winogradsky, apparently homologues of the globular bodies in the fusiform bacteria, which seem to be close relations of the spirochetes (Sanarelli, 1927). Upon the whole Lehmann and Neumann's classification seems by far the most logical and workable and has therefore been followed here.

Several attempts have been made to split *Actinomyces* into two or more genera. Wright (1905) would reserve the name *Actinomyces* for the anaerobic or microaerophilic organisms known to produce actinomycosis in man and cattle (the type of Wolff and Israel, 1891); for the aerobic forms he suggested the use of the name *Nocardia* (Trevisan, cit. after Buchanan, 1925). Haass (1906) would use the names *Actinomyces* for the aerobic and *Actinobacterium* for the anaerobic type,

and proposed to transfer those actinomycetes which produce a soft, bacterium-like growth, e.g. *Act. farcinicus* Nocard (1888), to the genera *Mycobacterium* and *Corynebacterium*. Pinoy (1913) followed Wright's division into an aerobic type, *Nocardia*, and an anaerobic type for which he substituted the name *Cohnistreptothrix* for *Actinomyces*. Wollenweber (1921) distinguished two subgenera under *Actinomyces: Aerothrix* with, and *Pionnothrix* without, aerial mycelium. A rational attempt to subdivide *Actinomyces* on the basis of definite morphological features was made by Ørskov (1923), who distinguished the following three groups:

I. Organisms which form a unicellular, non-septate vegetative mycelium, and an aerial mycelium composed of hyphae, thicker than those of the vegetative mycelium and dividing into spore-like bodies of regular and uniform size and shape. The name *Cohnistreptothrix* was suggested for this group.

II. Organisms in which the vegetative as well as the aerial mycelium divide by septa into pieces of irregular size and shape, without any spore-like bodies as in Group I. The aerial mycelium is absent in one subgroup, in which there is also a tendency to adopt the "angular" growth characteristic of the mycobacteria and corynebacteria which, it is contended, should really be included in this group, for which it was proposed to reserve the name *Actinomyces*.

III. Organisms which form a unicellular mycelium without aerial hyphae, but with spore-like bodies borne singly on the tips of short branches of the vegetative hyphae. It was proposed to call this group *Micromonospora*.

Ørskov's work marks a great step towards a better understanding of the natural relationships of the order we are dealing with, but his nomenclature (which, indeed, he himself only claims to be tentative) is less fortunate, since the name *Cohnistreptothrix* was first intended by Pinoy (1913) to be applied to anaerobic, pathogenic forms which, as Ørskov himself shows, at least partly belong to Group II, for which Ørskov would reserve the name *Actinomyces*. Further, Castellani and Chalmers (1919), who adopt Pinoy's nomenclature, give a list of not less than nine species of *Cohnistreptothrix* in the sense of Pinoy. An unreserved adoption of Ørskov's nomenclature, as regards Groups I and II, could, therefore, easily lead to confusion.

As mentioned above, the tendency among most of the earlier authors was to regard the actinomycetes as "pleomorphic bacteria". Sauvageau and Radais (1892), on the other hand, considered them true fungi of the genus *Oospora*, and Lachner-Sandoval (1898) would transfer *Actinomyces* as well as *Mycobacterium* and *Corynebacterium* to the *Fungi imperfecti* because of their branching growth, which was thought to be incompatible with the nature of bacteria. Notwithstanding the fact that *Actinomyces* has been included in nearly all classifications of bacteria, there has been a good deal of controversy whether these organisms should be regarded as "true bacteria" or fungi. In more recent time the tendency has mostly been to regard them as a special group of microorganisms, apart from both the fungi and the bacteria (Waksman, 1919; Lieske, 1921; Ørskov, 1923), and in most papers on the subject we meet the following statement, somewhat varied in its verbal expression: "The actinomycetes occupy an intermediate position between the bacteria and the fungi". Claypole (1913) regards them as an ancestral type of microorganism, giving rise, on one side to yeasts and higher filamentous fungi, and on the other to mycobacteria, corynebacteria, and ordinary bacteria. Lieske (1921) pointed out that there is a much wider gap between the actinomycetes and the simplest hyphomycetes (e.g. *Oidium lactis*) than between bacteria and

certain types of actinomycetes. Drechsler (1919) regarded the genus *Actinomyces* as entirely conforming with the hyphomycetes and devoid of any bacterial characteristics. It is obviously Drechsler's limitation of his fine morphological work to organisms of Ørskov's Group I, which has led him to this rather extreme view.

The present work represents a study of the morphology and biology of about 70 strains of *Actinomyces* isolated from various Australian soils, with the purpose of obtaining a more solid systematic basis for the study of this important group of soil microorganisms. Special attention was given to the work of Ørskov; the genus *Micromonospora* proposed by him has been made the subject of a preliminary account previously (Jensen, 1930*a*). The non-systematic term "actinomycetes" or "ray fungi" covers *Micromonospora* as well as the organisms of Ørskov's Groups I and II—filamentous organisms of a definitely mycelial growth, of bacterial dimensions, and generally producing an aerial mycelium. As the Order Actinomycetales we shall regard these organisms as well as the genera *Mycobacterium* and *Corynebacterium*, which, as most investigators agree, form a natural group of microorganisms.

Experimental.

The organisms included in this study were obtained partly from soil samples from lawns and flower beds in the grounds of Sydney University (here designated by the characters AI, AII, AIII, U, and G), partly from a number of samples in the collection of soils in the School of Agriculture, Sydney University; these latter are designated by the figures 6, 92, 125, 129, 130, 148, 163, and 176.

Isolations were in most cases carried out by plating on a dextrose-casein-agar medium previously described (Jensen, 1930*a*, 1930*b*). In a few cases soil was inoculated into a mineral nutrient solution with phenol or paraffin as source of energy, and platings were made therefrom, when a growth of microorganisms had taken place. The following media were used for studying the cultural characters of the organisms.

1. Saccharose agar.—Saccharose 20·0 gm.; $NaNO_3$ 2·0 gm.; K_2HPO_4 1·0 gm.; $MgSO_4$ 0·5 gm.; agar 15·0 gm.; water 1,000 c.c.

2. Dextrose agar.—Dextrose, 10·0 gm.; asparagin 1·0 gm.; K_2HPO_4 0·5 gm.; $MgSO_4$ 0·5 gm.; agar 15·0 gm.; water 1,000 c.c.

3. Nutrient agar.—Meat extract 5·0 gm.; peptone 10·0 gm.; dextrose (in some cases glycerin) 10·0 gm.; agar 15·0 gm.; water, 1,000 c.c.

4. Dextrose broth.—Same, without agar.

5. Potato.—6. Milk.—7. Gelatin (15% gelatin in tap water, pH 7·0).

Diastatic activity was tested on agar containing 1% soluble starch, 0·2% $NaNO_3$, 0·1% K_2HPO_4, and 0·05% $MgSO_4$. A corresponding mineral solution was used, with strips of filter paper for testing the power of decomposing cellulose, and with flakes of paraffin wax for determination of ability to utilize paraffin. Invertase-production was tested in a solution corresponding to medium 1. Resistance to hydrogen-ion concentration was tested by measuring the reaction (colorimetrically) in a physiologically acid nutrient solution (dextrose 2·0%, NH_4Cl 0·2%, K_2HPO_4 0·02%, $MgSO_4$ 0·05%) after three weeks' incubation. With some of the strains, a study of the ability to utilize various sources of carbon (pentoses, mono- and disaccharides, higher alcohols) was carried out. The above-mentioned solution with $NaNO_3$ and mineral nutrients was used in this case, where tests were also made for the reduction of nitrate to nitrite. A complete set of experiments with all the strains was not carried out, since there is little hope of obtaining any distinct species differen-

tiation by means of these tests (Waksman, 1919; Jensen, 1930*b*). All cultures were incubated at 28–30° C., unless otherwise stated, at which temperature they made an excellent growth. For morphological studies the dextrose-casein-agar (in the following simply called casein agar) was mostly used; the thin, spreading growth produced by most organisms on this medium makes it well adapted for direct microscopical study of the growth on open agar blocks (Ørskov, 1923) by means of high-power dry lenses. This was found the only quite satisfactory method for studying the aerial mycelium in its natural arrangement, although good results, particularly for photography, were also obtained with a simplification of the method of Drechsler (1919): a clean cover slip is pressed gently against the aerial mycelium, lifted without any lateral sliding, the adhering material is fixed with formalin vapour, dried in the air, and stained with dilute carbol fuchsin.

General Morphology and Biology.

As a result of these studies, it was found possible to divide the actinomycetes into two main groups, essentially agreeing with Ørskov's division; in one of them, two subgroups could be distinguished:

I. Spores are formed in an aerial mycelium.
   A. Substratum mycelium remains undivided.
   B. Substratum mycelium divides into a kind of "fragmentation spores".
II. No spores are formed in the aerial mycelium. Substratum mycelium divides usually into more or less bacteria-like elements.

Group I A is identical with Ørskov's Group I. The characteristic feature of these organisms is the following course of development: when transferred to a suitable medium, the aerial spores germinate with the production of a filament, usually about 0·4–0·6 μ thick, which rapidly forms monopodial branches and develops into an extensive mycelium, which forms a colony of a characteristic firm and cartilaginous consistence. The mycelium remains nonseptate and coherent even in very old cultures—months and even years—falling to pieces only as a result of local processes of degeneration in the hyphae (Foulerton and Jones, 1902; Ørskov, 1923), which usually retain their uniform thickness. Only in a single one of the species of this group studied here is there a tendency to formation of peculiar swollen cells in some media (Pl. xix, fig. 2). A few words should here be said on the mode of formation of the mycelial branches. In all the actinomycetes, of this as well as of the two other groups, we see the first beginnings of the branches arise as quite small, slightly oblong or pear-shaped external buds, attached to the main stem by very thin stalks. The bud generally grows and stretches out into a long hyphal branch, which for a considerable time may be separated from the main stem by a basal constriction (Pl. xix, figs. 1, 3, 8; Pl. xx, figs. 18, 22). This phenomenon, which has been commented upon by comparatively few authors (e.g. Goadby, 1903, and Fennel, 1918), may possibly have something to do with the alleged existence of filterable forms in actinomycetes (Leyton and Leyton, 1916; Lucksch, 1930). The initial buds are sometimes, for instance, in the organisms of Group I B in liquid media, so small as to come near to the limit of visibility, and it is not inconceivable that they may be small enough to pass through bacterial filters, if they are torn loose from the cells that produce them.

After the formation of this "vegetative" or "substratum" mycelium, follows sooner or later a formation of aerial mycelium, where the growth is in contact with the air. The aerial hyphae, which are at first visible as small refractive

granules outside the vegetative hyphae, are constantly thicker than these, of very variable length, and more or less branched. The end branches are often coiled into more or less regular spirals and sometimes situated in crown- or whirl-like arrangements. The abundance of the aerial mycelium varies greatly both with the organism and the medium. In rich media, e.g. nutrient agar, it is often altogether absent, and in some strains it is very scant in all media, though always present in some medium or other, especially such as allow only a limited growth (cf. Waksman, 1919, and Ørskov, 1923). After some time, the end branches of the aerial hyphae divide basipetally into regular-sized elements, spherical to elliptical, or in some cases cylindrical. When seen under a high-power dry lens, the aerial hyphae exhibit a characteristic beaded appearance, due to small opposite incisions in the cell walls, connected by fine transversal lines. In stained preparations these lines appear as unstained intervals separating the elements into which the contents of the hyphae have divided. These unstainable intervals may even be seen before any external changes in the hypha have taken place; it seems thus, that the protoplasm separates without any formation of primary transversal walls, as contended by Ørskov (1923) and most other authors on this subject. It has not been found possible to decide whether there is actually a formation of primary septa, which afterwards split in halves, in favour of which Drechsler (1919) has brought forth strong evidence. The elements, into which the aerial hyphae divide, represent spores of the organisms, as the term "spores" is defined by Ørskov (1923): "Bodies that are identical to one another in form, which have a special mode of formation, and are distinguished by a greater power of resistance than the mycelial filaments, and which, under adequate conditions, grow out into a new mycelium". It is important to realize that they are spores in the mycological sense of the word, viz., reproductive bodies, and that they have nothing to do with the endospores of certain bacteria (the genera *Bacillus* and *Clostridium*). Numerous authors have pointed out that the aerial spores of the actinomycetes possess a somewhat higher thermoresistance than the vegetative mycelium. This was also found to be the case here. While the vegetative mycelia were usually killed in 2–5 minutes at 60° C., the spores would generally survive for 10 minutes at 60° C. or 2–5 minutes at 70° C.

This group comprises the great majority of soil actinomycetes. Hereto belong all the organisms described by Krainsky (1914), Waksman and Curtis (1916), Waksman (1919), Drechsler (1919), Millard and Burr (1926),* and further *Act. pheochromogenus* Conn (1919), *Act. cloacae* Brussoff (1920), and the organisms described by the present author in two earlier papers (Jensen, 1928, 1930*b*). On the other hand, the group comprises apparently very few animal pathogens. The only unquestionably pathogenic species seems to be *Act. madurae* (Vincent, 1894; Ørskov, 1923). The classical *Act. hominis* Bostroem (1890), as well as forms isolated by Henrici and Gardner (1921) and Grubauer (1925), do certainly belong to this group, but their pathogenicity seems somewhat doubtful.

Group I B.—This group resembles the previous one perfectly with regard to the formation of aerial mycelium and the mode of spore formation herein (Pl. xix, figs. 6, 7), but differs from it in a characteristic process of division of the vegetative hyphae, giving rise to bodies of somewhat varying appearance, but often similar to the aerial spores. This phenomenon does not appear in the same fashion in all

* With the possible exception of two species, *Act. maculans* and *Act. salmonicolor*, which might seem to belong to other groups.

strains or in all media. On nutrient agar the hyphae show, after a few days' growth, a number of fine transversal lines, appearing as unstainable intervals in stained preparations, and separating the hyphae into rod-shaped bodies of somewhat unequal size (Pl. xix, fig. 8). In some cases these bodies remain at a stage resembling diphtheroid rods (Pl. xix, figs. 11, 12), in others they are very nearly coccoid. Sometimes they resemble the somewhat mysterious objects which Lieske (1921) describes under the name of "four-hyphae-spores" (Vierhyphensporen). Similar forms arise in broth and dextrose-asparagin-solution and on potato, in which latter medium one strain (6 VI) produces fairly large (1·2–1·5 μ), roughly globular bodies, apparently arising by lateral budding from the hyphae (Pl. xix, fig. 10); this represents probably a modification of the normal lateral branches as described above under Group I A. When these globular to rod-shaped bodies are transferred to a suitable medium (dextrose or casein agar, sometimes potato extract agar) they produce a largely undivided vegetative mycelium and an aerial mycelium with spores as in Group I A (Pl. xix, figs. 6, 7). Besides this, one particular strain (6 VI) produces in acid dextrose-NH_4Cl-solution an abundance of big, globular bodies, arising by division and subsequent swelling of the hyphae (Pl. xix, fig. 9). When transferred to fresh media, these bodies have regularly failed to show any further development. Although they look like what have been described as "gonidangia" in numerous bacteria it might, in view of their apparent incapacity of growth, seem likely that they are really "involution forms" in the true sense of this much-misused term: aberrant cell types arising under subversive conditions of growth (in this case, where the growth has been checked through acidification of the medium) and devoid of any capacity of further development.

These observations throw an interesting light on a phenomenon which has caused a good deal of controversy, namely, the so-called "fragmentation spores" or "oidiospores" of the actinomycetes. A phenomenon termed "fragmentation" was first described by Boström (1890) in his *Act. hominis*, but later explained by Sauvageau and Radais (1892) as an artefact due to uneven staining of the hyphae. Lachner-Sandoval (1898) distinguished between "segmentation", i.e. spore formation in the aerial hyphae, and "fragmentation", i.e. division of the vegetative hyphae into pieces of more uneven length. Neukirch (1902) added a third kind of "spores", called "oidiospores", said to be produced by formation of primary transversal walls in the vegetative hyphae. These phenomena have been little studied in recent time, and the interpretation of the earlier records is often difficult, as pointed out by Ørskov, since there is no discrimination between the present Group I and the subsequent Group II, in which division of the vegetative hyphae is a prominent feature. Brussoff (1919) explained the fragmentation in the same manner as Sauvageau and Radais, namely, as due to the presence of deeply staining granules of volutin in the hyphae; the only organism studied was a typical representative of Group I A. Lieske (1921) describes and figures "oidiospores" in the sense of Neukirch, but the strain in question seems to be one of Group II. Foulerton and Jones (1902) describe a process of "fragmentation" of the vegetative hyphae of actinomycetes which formed spores in the aerial mycelium; here, however, it seems to be a case of local degeneration in the hyphae, as described by Ørskov (1923) in organisms of Group I. Still, even when we allow for these two sources of error—failure to distinguish between the Groups I and II, and the phenomena of degeneration—there remains a phenomenon which resembles the spore formation in the aerial mycelium, namely, the division of the vegetative hyphae into short rods and cocci of fairly regular size and shape, such

as we meet it in the organisms which we have here classified as Group I B. One of the organisms studied by Ørskov (1923) and included by him in his Group I, although with reservation, viz., *Act. Affanassiew*, behaved similarly; indeed, his figure of this organism resembles perfectly our Fig. 13, Pl. xix.

Organisms of this group seem to be comparatively rare. In the present study, only three strains were found. There are not many records in the literature of organisms which can be recognized as belonging hereto, except *Act. Affanassiew* referred to above. The "*Streptothrix*" *buccalis* described by Goadby (1903) and an organism studied by Bachmann (1922) are certainly of this type, and the same seems to be true of a thermophilic actinomyces studied by Sames (1900) and one of the thermophilic actinomycetes studied by Schütze (1908).

Besides the outstanding morphological character of spore formation in the aerial mycelium, the organisms of Groups I A and I B have a number of biological features in common, which distinguish them from Group II. All of them liquefy gelatin, although with widely varying rapidity, as also pointed out by Waksman (1919) and Ørskov (1923). Further, they are all capable of exerting diastatic action on starch, as previously noted by Waksman (1919); the only organism studied by him, which did not liquefy gelatin or hydrolyze starch (*Act. asteroides*) belongs to Group II.* Several organisms of this group produce a characteristic brown pigment in protein media and a black pigment on potato, and not a few of them are capable of decomposing cellulose.

Group II corresponds to Ørskov's Groups II A and II B. The course of development is as follows: initially a more or less extensive vegetative mycelium is formed, consisting of hyphae similar to those in Group I. Sooner or later the vegetative hyphae divide into segments of rather varying size and shape, sometimes swollen and irregular, but usually resembling mycobacteria or corynebacteria; the process of segmentation often goes so far as to produce quite coccus-like forms. After these bacteria-like elements have been formed by division of the initial mycelia, they continue to multiply in the manner characteristic of the mycobacteria and corynebacteria, which Ørskov (1923) describes as "angular growth". This development is, however, subject to very great variations, depending both on the organisms and the medium. In some cases, e.g. the *Proact. flavescens* described below, the angular growth is hardly noticeable; the mycelia seem to remain at the stage of septation, as in Group I B, and even seem to remain undivided in some media. In another organism, described in detail in an earlier paper (Jensen, 1931*a*), variants arise with a constantly undivided vegetative mycelium as in Group I A. Finally, in the *Proact. paraffinae* described below we do not find any angular growth, but a division of the hyphae into round to oval bodies, thicker than the undivided filaments and resembling the aerial spores of Group I. An aerial mycelium is generally formed at an early stage of the growth; its hyphae are generally simpler, shorter and less branched than those of Group I, and never forming spirals. As Ørskov points out, the aerial hyphae are of the same thickness as those of the vegetative mycelium, and a differentiation into spores, as in Group I, does not take place here. In stained preparations, or when a coverslip is placed upon the growth, the aerial hyphae fall apart into generally rod-shaped fragments of variable length, and they do not show any higher

* Non-diastatic organisms of Group I may occur, such as certain organisms described by Henrici and Gardner (1921) and Millard and Burr (1926), but they are decidedly exceptional.

thermoresistance than the vegetative hyphae. It is undoubtedly this distinctive character of presence or absence of spore formation in the aerial mycelium, which should be considered the most important criterion for the distinction between Groups I and II, rather than (as Ørskov thinks) the division of the vegetative mycelium, which is subject to such wide variation, and which also occurs in Group I B. Ørskov's distinction between his groups II A and II B was based on the formation of aerial mycelium in Group II A and absence of this, in addition to a marked tendency to angular growth, in Group II B, but he is right in admitting that this difference is not very profound, since the property of forming aerial mycelium seems easily to be lost. *Act. farcinicus* and *Act. polychromogenes*, which Ørskov found without aerial mycelium, did apparently form this at the time of their first isolations (Nocard, 1888; Vallée, 1903). All the present organisms of Group II, except one, produced more or less aerial mycelium, although sometimes visible only under the microscope.

Besides these morphological characters, the organisms of Group II differ from Group I in other ways. In distinction from Group I, many of them are incapable of liquefying gelatin or hydrolyzing starch. On the other hand, most of them are capable of utilizing paraffin, which few of Group I can do. None of them is capable of decomposing cellulose, and they do not form any brown pigment in protein media or black pigment on potato. Finally, some of the forms studied here are acid-fast, like numerous pathogenic actinomycetes (*Act. asteroides, farcinicus, caprae*, and others) of this group. The statement of Ørskov (1923), that the organisms of Group II always produce an early surface growth in liquid media, could not be confirmed in all cases.

As will be seen from the above, and, as Ørskov points out, this group is a good deal more heterogeneous than Group I. The *Proact. flavescens* described below comes rather close to Group I both in morphological and biological respect; *Proact. agrestis*, on the other hand, shows the closest possible approximation to the mycobacteria: its initial mycelia are very small (Pl. xx, fig. 17), it assumes rapidly an entirely bacterium-like appearance (Pl. xx, fig. 19), it produces a kind of "smooth" and "rough" variant like many bacteria, and it produces an actual turbidity in liquid media, which the actinomycetes are supposed never to do. Indeed, this organism was first described as *Mycobacterium agreste* by Gray and Thornton (1928). As a matter of fact, a sharp line of distinction has never been drawn between *Actinomyces* on one side and *Mycobacterium* and *Corynebacterium* on the other. Acid-fastness occurs among actinomycetes as well as among mycobacteria, and serological reactions have failed to give any distinct separation (Fritsche, 1908; Claypole, 1913). On the morphological side, the sometimes quite profuse branching in mycobacteria and corynebacteria and the slightly accentuated mycelial character of such forms as *Act. farcinicus* and the anaerobic pathogenic actinomycetes (the Wolff-Israel-type) tend to obscure the limit. Ørskov (1923) is of the opinion that the corynebacteria and mycobacteria should really be included in Group II of the actinomycetes, because their manner of cell division (angular growth) is exactly the same. It would seem, however, that a certain distinction can be made. As Haag (1927) points out, the cell division begins in the mycobacteria and corynebacteria at so early a stage that a small mycelium is only occasionally formed (cf. the studies of Miehe (1909) on *Myc. tuberculosis* and of Ørskov (1923) on *Myc. phlei*). It is therefore suggested that the constant formation of an initial mycelium be regarded as a criterion for distinguishing the actinomycetes of Group II from the mycobacteria and corynebacteria. On the

basis of this we must regard *Proact. agrestis*, which constantly forms a small initial mycelium, as belonging to the actinomycetes, whereas for instance *Myc. coeliacum* (Gray and Thornton, 1928), which forms a small mycelium only occasionally (Jensen, 1931*b*) should remain in the genus *Mycobacterium*.

This group includes only few soil organisms. The organism described by Beijerinck as *Bac. oligocarbophilus* has been shown by Lantsch (1922) and Kober (1929) to be an actinomycete, undoubtedly belonging to Group II. Several others have been described as mycobacteria; this is true of at least two of the phenol-decomposing organisms described by Gray and Thornton (*Myc. agreste* and *Myc. actinomorphum*, as shown below) and probably also, as pointed out by Haag (1927), of the organisms studied by Vierling (1921). Beijerinck (1914) described a soil organism to which he gave the name *Actinococcus cyaneus*; his description and figures leave no doubt that it is an organism closely related to *Proact. agrestis* (see below). The generic name *Actinococcus*, however, is invalid (Buchanan, 1925). On the other hand, the group includes such typically pathogenic forms as *Act. farcinicus* and *Act. asteroides* (Ørskov, 1923). Besides this, a survey of the medical literature shows a large number of cases where organisms which seem to belong to this type, have been isolated from actinomycotic affections in man and animals. It is, however, in most cases difficult to see whether the organisms really belong to Group II or to Group I B, because they have usually only been cultivated and studied in complex organic media (nutrient agar, potato, broth, gelatin, serum, etc.), and Group I B will usually only show its characteristic spore formation in synthetic media. The anaerobic or microaerophilic, short-hyphed actinomycetes most commonly encountered in cases of actinomycosis (Wolff and Israel, 1890; Wright, 1905; Harbitz and Grøndahl, 1911; Dresel, 1915; Lieske, 1921; Magnusson, 1928, and others) demand a special interest. Ørskov (1923) studied an organism of this type and found it belonging to Group II. There is no proof, however, that this is true of all organisms of this type, since organisms of Group I B may also be concerned. Gasperini (1895) mentions that *Act. bovis*, an organism of Group I, may produce "fragmentation spores" by growth under reduced oxygen tension and Morelli (1930) claims to have changed long-hyphed, aerobic actinomycetes into organisms of the Wolff-Israel type by gradual adaptation to anaerobic conditions. In agreement herewith it was found in the present case, that *Act. albus* and *Act. californicus*, when grown on nutrient agar under reduced oxygen tension (in tubes in air-tight connection with cultures of a rapidly growing, aerobic soil mycobacterium), produced a vegetative mycelium in the condensation water, which showed a tendency to break up into rather short, rod-shaped pieces similar to Group I B. It might thus seem that there is no sharp distinction between Groups I A and I B, and that the short-hyphed, anaerobic, pathogenic actinomycetes may belong to Group I as well as to Group II. Mertens (1903) and Lieske (1921) observed a gradual change of anaerobic, short-hyphed forms into aerobic, long-hyphed ones, wherein Lieske seeks an explanation for the apparent contradiction between the results of Boström (1890) and Wolff and Israel (1891). These may also be cases of changes between Groups I A and I B, and it has in no instance been proved that Group I may change into Group II, or vice versa.

A Suggestion for Classification of the Order Actinomycetales.

It would doubtless be right, as Ørskov (1923) proposes, to regard Groups I and II as two distinct genera, since there is admittedly a very wide difference

between the quite fungus-like organisms of Group I A and the most bacterium-like forms of Group II, but his nomenclature is, as previously pointed out, less fortunate. In the present writer's opinion it would be better to reserve the name *Actinomyces* for Group I, since the first adequately described organism of this group was *Act. hominis* Boström (1890). For Group II one might suggest the name *Proactinomyces*, to be included in the family Proactinomycetaceae (Lehmann and Neumann, 1927). We can now construct the following modification of Lehmann and Neumann's classification of the order Actinomycetales:

A. No spores are formed ..... Family Proactinomycetaceae.
  I. No mycelium is formed.
    a. Acid-fast organisms* ..... Genus *Mycobacterium.*
    b. Non-acid-fast organisms* ..... Genus *Corynebacterium.*
  II. Mycelium is formed ..... Genus *Proactinomyces.*
B. Spores are formed ..... Family Actinomycetaceae.
  I. Spores in aerial mycelium ..... Genus *Actinomyces.*
  II. Spores terminally on branches of vegetative mycelium .....
  ..... Genus *Micromonospora.*

The genus *Mycoplana* Gray and Thornton (1928) may possibly be placed as a genus parallel to *Mycobacterium* and *Corynebacterium.*

As we have repeatedly pointed out, the transitions between all these genera and families are quite gradual, so that we find a continuous sequence leading from the mycobacteria and corynebacteria to the most highly differentiated, entirely mould-like forms of *Actinomyces*, Group A (*Act. viridochromogenus*, *Act. reticuli*, etc.). While thus the evidence of connection with the "true" bacteria is very complete, there seems to be no connection with any genus of the Eumycetes. We must, therefore, regard the genus *Actinomyces* as a highly developed and specialized form of bacteria (cf. Lantsch, 1922), and it is not justified to speak of the actinomycetes as "a connecting link between bacteria and fungi", at least if we attach any suggestion of a phylogenetic relationship to this phrase.

Description of various Groups of Soil Actinomycetes.

Owing to the marked variability and the abundance of transition forms in the actinomycetes, the term "species" is in the following used in the sense of Waksman's "species groups", i.e. broad groups of strains agreeing in certain outstanding morphological and biological features. The opposite practice, adopted by Millard and Burr (1926), of establishing species differentiations on the basis of every observed constant difference, is certainly logical, but hardly practicable, since nearly every strain of actinomycetes isolated from a plating from an ordinary soil could then be raised to the rank of species.

Genus Actinomyces, Group A.

Actinomyces albus (?) Krainsky (1914).

Three strains. *Hab.*—Soils 130, 163, G.—They seem to agree better with the description of Krainsky than with that of Waksman (1919). A fourth strain differed from them mainly in the formation of a pink pigment in most media.

Morphology.—The vegetative mycelium is of the usual type. The aerial mycelium consists of long, tangled, not very much branched hyphae, 0·4–0·5 μ

* This conventional way of distinguishing between *Mycobacterium* and *Corynebacterium* will probably on closer study be found to require some revision (cf. Gray and Thornton, 1928, and Jensen, 1931*b*).

thick, appearing homogeneous and undivided when examined in undisturbed condition. Only in strain G do a few hyphae on dextrose agar show the characteristic beaded appearance which accompanies the spore formation in *Actinomyces*. When mounted in water or studied in impression preparations, the aerial hyphae fall apart into rod-shaped pieces of the same thickness as the aerial hyphae, and of somewhat variable length, 0·4–0·5 × 2–4 μ, with a tendency to bipolar staining (Pl. xix, fig. 5). In strain G, some of these rods are thicker (0·7–0·8 μ) than the undivided filaments. The mode of spore formation is similar to that described by Drechsler (1919) for his strain XIII. It is also of interest to note that Ørskov (1923) mentions that the typical spore formation is difficult to detect in actinomycetes with cylindrical spores, e.g. "*Leptothrix*" *buccalis*. Only in strain G did the spores show a higher thermoresistance than the vegetative hyphae. We must, therefore, since the spore formation is so little typical, regard this species-group as situated at that end of the whole group I A, which shows the closest approximation to Group II (*Proactinomyces*).

Cultural characters.—Saccharose agar: Fair growth, becoming abundant; vegetative mycelium flat, spreading, becoming raised, first cream-coloured, later light ochre-yellow. Aerial mycelium abundant, smooth, cottony, pure white. Pale-yellow soluble pigment. Dextrose agar: Excellent growth, much resembling the previous. Nutrient agar: Excellent growth. Vegetative mycelium raised and wrinkled, with cracking surface, cream-coloured to straw-yellow. Aerial mycelium rather scant, white. Potato: Fair growth. Vegetative mycelium raised and wrinkled, cracking, dirty cream-coloured. Traces of white aerial mycelium.

Actinomyces aureus (?) Waksman and Curtis (Waksman, 1919).

Two strains. *Hab.*—Soils 163 and 176.

Morphology.—Aerial mycelium consists of long, tangled and branching hyphae; spirals not very numerous, short, sinistrorse. Spores nearly spherical, 1·0–1·2 μ.

Cultural characters.—Saccharose agar: Growth scant in one strain, good in one. Vegetative mycelium spreading, white. Aerial mycelium first scant, white, later abundant, crusty, greyish-brown with white edges. Dextrose agar: Fair to good growth. Vegetative mycelium raised, superficial, first cream-coloured, later deep-orange. Aerial mycelium well developed, dusty, first white, later greyish-brown. Nutrient agar: Excellent growth. Vegetative mycelium superficial, wrinkled, yellowish-grey. Aerial mycelium absent or traces of white. Deep-brown pigment. Potato: Vegetative mycelium raised, spreading, yellowish-grey, becoming nearly black. Traces of white aerial mycelium. Black pigment.

Actinomyces bobili Waksman and Curtis (Waksman, 1919).

One strain. *Hab.*—Soil 92.

This strain corresponds in most points to Waksman's description, although its aerial mycelium on saccharose and dextrose agar seems less scanty, and a heavy, brownish-black growth is produced on potato.

Actinomyces californicus Waksman and Curtis (Waksman, 1919).

One strain. *Hab.*—Soil 92. Another, less typical strain, probably belonging to the same group, was isolated from soil 163. Strain 92 agrees very well with Waksman's description.

ACTINOMYCES EXFOLIATUS Waksman and Curtis (Waksman, 1919).

Two strains. *Hab.*—Soil 176. Both strains correspond fairly well to Waksman's description. The colours are generally darker, and the initially white aerial mycelium on saccharose agar assumes in older cultures a rose-brown colour. Waksman states that the tendency of the growth on saccharose agar to crack and peel off, from which the specific name is derived, is easily lost; with the present strains it was not noticeable at all.

ACTINOMYCES FLAVUS (?) Krainsky emend. Waksman and Curtis (Waksman, 1919).

Four strains. *Hab.*—Soils 163 and 176.

Morphology.—Aerial hyphae long, tangled, with none or a few short spirals. Spores long oval, 0·8–1·0 × 1·0–1·5 μ.

Cultural characters.—Saccharose agar: Fair to good growth, one strain very scant. Vegetative mycelium heavy, superficially spreading, first white to cream-coloured, later ochre-yellow. Aerial mycelium thin, white to yellowish-grey, absent in one strain. Dextrose agar: Good growth. Vegetative mycelium heavy, superficially spreading, ochre-yellow. Aerial mycelium thin, in patches yellowish-white to ash-grey. Nutrient agar: Excellent growth. Vegetative mycelium raised, wrinkled, yellowish-brown. Aerial mycelium absent or trace of white. Brown pigment. Potato: Good growth. Vegetative mycelium raised, lichnoid, yellowish-brown to greenish-olive. Black pigment.

ACTINOMYCES FULVISSIMUS Jensen (1930*b*).

Two strains. *Hab.*—Soil AII, and acid sand soil from Cooper Park. The strains generally agree with the author's previous description, but the golden pigment is less typical in saccharose agar, and the aerial mycelium more abundant and pure grey on dextrose agar.

This species is probably identical with *Act. flavus* Millard and Burr (1926); this name, however, is obviously invalid, since this organism is well distinguished from the one to which Krainsky previously (1914) had given the name *Act. flavus* (see above).

ACTINOMYCES GRISEUS Krainsky emend. Waksman and Curtis (Waksman, 1919).

One strain. *Hab.*—Soil U. It agrees well with Waksman's description, from which it differs only in producing a lemon-yellow soluble pigment in nearly all media. *Act. griseus* is one of the most easily recognized species of actinomyces, as well as one of the most well-defined, as can be seen from the closely tallying descriptions by Waksman (1919), Fellers (1922), and the present author (Jensen, 1930*b*).

ACTINOMYCES 218, Waksman (1919).

One strain. *Hab.*—Soil 6. This strain corresponds almost perfectly to Waksman's description of his strain 218, which seems to be a "chromogenic" parallel to *Act. griseus*, from which it differs only in producing a brown pigment in protein media and in being less strongly proteolytic.

ACTINOMYCES HYGROSCOPICUS, n. sp.

Seven strains. *Hab.*—Soils 92, 163, and 176.

Morphology.—Vegetative mycelium of the usual type; the hyphae are rather stout, 0·6–0·8 μ thick. Aerial hyphae long, tangled, richly branched, 0·8–1·0 μ

thick; spirals are numerous, sinistrorse, narrow, sometimes long, but mostly quite short, only 1–2 turns, closed, typically situated as dense clusters on the main stems of the aerial hyphae. Spores oval, 0·8–1·0 × 1·0–1·2 μ.

Cultural characters.—It is a striking feature in this species-group, that the aerial mycelium, which in other actinomycetes is strikingly hydrophobic, does here in cultures on certain media—dextrose or glycerin asparagin agar—become moistened and exhibits dark, glistening patches which, when touched with the needle, prove to be a moist, smeary mass of spores. This characteristic feature is not equally distinct in all strains.

Saccharose agar: Good to abundant growth. Vegetative mycelium heavy, superficially spreading, folded, glossy surface, first white to cream-coloured, later sulphur-yellow to yellowish-grey, with golden to light-orange reverse. Soluble pigment of the same colour. Aerial mycelium scant, thin, white, or altogether absent. Dextrose agar: Good growth. Vegetative mycelium superficially spreading, surface granulated, first cream-coloured to straw-yellow, later dull chrome-yellow to brownish-orange. Aerial mycelium thin, smooth, dusty, white to pale yellowish-grey, after 1–2 weeks more or less abundantly interspersed with small, moist, dark violet-grey to brownish patches which gradually spread over the whole surface. Light-yellow soluble pigment. Nutrient agar: Good growth. Vegetative mycelium raised, wrinkled, glossy, first cream-coloured, later yellowish-grey with yellowish-brown reverse. Aerial mycelium mostly absent, sometimes scant white. Potato: Fair growth. Vegetative mycelium raised, wrinkled, first cream-coloured, later yellowish-grey to dull-brownish. Aerial mycelium absent or trace of white.

Biochemical features.—Saccharose is inverted. Nitrate is not reduced with saccharose as source of energy. Starch is hydrolyzed. Cellulose is decomposed readily by some strains. Gelatin is slowly liquefied without any pigment formation. Milk is completely digested in 3–4 weeks at 30° C., without any previous coagulation; the reaction becomes faintly acid (pH about or below 6·0).

Actinomyces microflavus (?) Krainsky (1914).

One strain. *Hab.*—Soil 176.

Morphology.—Aerial mycelium consists of tufts of short, straight hyphae, not much branched, no spirals. Spores oval, 1·0–1·2 × 1·2–1·5 μ.

Cultural characters.—Saccharose agar: Very scant growth. Vegetative mycelium spreading widely into the medium, thin, colourless. Aerial mycelium thin, pale reddish-brown. Dextrose agar: Scant growth. Vegetative mycelium forms isolated colonies, first colourless, becoming deep ochre-yellow. Aerial mycelium thin, yellowish-grey with rose spots. Nutrient agar: Fair growth. Vegetative mycelium flat, growing down, cream-coloured, central part raised, folded, ochre-yellow. Aerial mycelium thin, white, limited to the flat part of the growth. Potato: Slow, but eventually good growth of a very characteristic appearance. Vegetative mycelium first flat, spreading into medium, yellowish with white edges. After 10–15 days the central part appears raised, folded, mulberry-like, pure ochre-yellow. Trace of white aerial mycelium.

Actinomyces parvus (?) Krainsky (1914).

Three strains. *Hab.*—Soils 125, 176, and U. Otherwise of very common occurrence in Australian soils.

Morphology.—Vegetative mycelium of strain U shows in culture on nutrient agar a tendency to formation of remarkable, big, globular to pear-shaped bodies

(Pl. xix, fig. 2). Aerial hyphae fairly short, straight, with little branching. Spores oval, 0·8–1·0 × 1·0–1·2 μ.

Cultural characters.—Saccharose agar: Scant growth. Vegetative mycelium flat, growing down, first colourless, later straw-yellow. Aerial mycelium absent or thin white veil. Dextrose agar: Scant growth. Vegetative mycelium slightly raised, wrinkled, sulphur-yellow to honey-yellow. Aerial mycelium absent or trace of white. Nutrient agar: Fair growth. Vegetative mycelium raised, lichnoid, first honey-yellow, later rust-brown, of a somewhat soft consistence. No aerial mycelium. Potato: Scant growth. Vegetative mycelium raised, much wrinkled, first cream-coloured, later dirty honey-yellow to olive-yellow. No aerial mycelium.

Krainsky states that his *Act. parvus* was strongly proteolytic. The present strains showed this property only to a slight extent.

ACTINOMYCES RETICULI Waksman and Curtis (Waksman, 1919).

Two strains. *Hab.*—Soil 176. Waksman states that strains of this species may vary widely in their cultural characters. The present strains agreed with Waksman's description in the peculiar structure of the aerial mycelium, although they differed in several other respects.

Morphology.—Aerial mycelium consists of long, richly branching hyphae, 0·8–1·0 μ thick. The branches are arranged in definite whirls, but terminate in short, sinistrorse spirals, unlike the organism described by Waksman. Spores are oval, 0·8–1·0 × 1·2–1·6 μ.

Cultural characters.—Saccharose agar: Scant growth. Vegetative mycelium thin, spreading, colourless. Aerial mycelium thin, smooth, slate-grey. Dextrose agar: Good growth. Vegetative mycelium flat, growing down, white. Aerial mycelium abundant, smooth, lead-grey. Nutrient agar: Good growth. Vegetative mycelium raised, wrinkled, yellowish-brown. Trace of white aerial mycelium. Deep-brown pigment. Potato: Good growth. Vegetative mycelium raised, wrinkled, greyish-black. Trace of white aerial mycelium. Black pigment.

ACTINOMYCES ROSEOCHROMOGENUS (Krainsky).

Synonym.—*Act. roseus* (Krainsky) emend. Waksman and Curtis (Waksman, 1919).

The use of the name *roseus* by Krainsky (1914) can hardly be regarded as valid, since it was used previously by Namyslowski (1912) for an organism which was apparently different from the one studied by Krainsky. It is, therefore, suggested to replace the specific name by *roseochromogenus*.

Morphology.—Aerial hyphae long, not very much branching, 1·0–1·2 μ thick. The branches terminate in fairly long, regular, sinistrorse spirals. Sometimes 3 to 5 branches are seen issuing together from the end-point of a main stem, thus giving a suggestion of whirl formation as in *Act. reticuli*.

Cultural characters.—Saccharose agar: Scant growth in one strain, abundant in one. Vegetative mycelium flat, spreading, colourless to white with pale-yellow reverse. Aerial mycelium abundant, smooth, pale greyish-rose. Dextrose agar: Good growth. Vegetative mycelium smooth, spreading, pale-yellow. Aerial mycelium abundant, smooth, cottony, first white, after a few days becoming rose-cinnamon, with many small white tufts. Nutrient agar: Excellent growth. Vegetative mycelium spreading, wrinkled, first yellowish-grey, later red-brown. Aerial mycelium develops late, first white, then pale rose-grey. Deep-brown pigment. Potato: Excellent growth. Vegetative mycelium spreading, roughly

granulated, yellowish-grey to greyish-black. Aerial mycelium absent or trace of white. Black pigment.

Dissociation.—By plating from the tufts of white aerial mycelium arising on dextrose agar or casein agar, a variant with pure white aerial mycelium is obtained.

Actinomyces rutgersensis (?) Waksman and Curtis (Waksman, 1919).

Two strains. *Hab.*—Soils 6 and A II.

Morphologically these strains differ from the organism described by Waksman in not producing real spirals; the aerial hyphae are only curled and waved to a considerable extent. The cultural characters agree very well with those listed by Waksman.

Actinomyces verne (?) Waksman and Curtis (Waksman, 1919).

Two strains. *Hab.*—Soils 125 and 163.

Morphology.—Aerial mycelium on dextrose agar consists of much curled and tangled hyphae, with irregular, sinistrorse spirals, 0·8–1·0 μ thick. Spores short cylindrical, 1·0 × 1·2–1·6 μ, not formed until after about three weeks; also many curved fragments, 6–10 μ long, are formed.

Cultural characters.—Saccharose agar: Good growth. Vegetative mycelium superficially spreading, slightly raised, first pale olive-yellow, later dark olive-grey to brownish-grey. No aerial mycelium. Yellowish to olive-brown soluble pigment. Dextrose agar: Good growth, similar to previous, with small tufts of whitish to greenish-grey aerial mycelium. Nutrient agar: Fair growth. Vegetative mycelium raised, wrinkled, cream-coloured to yellowish-grey. No aerial mycelium. Brown pigment in one strain. Potato: Fair growth. Vegetative mycelium wrinkled, greenish-grey to brownish-black. Trace of white aerial mycelium. Brownish pigment.

Actinomyces viridochromogenus (Krainsky) emend. Waksman and Curtis.

Three strains. *Hab.*—Soils 163, 176, U. Otherwise very common in Australian soils. The present strains differ a good deal from the description by Waksman in their manner of growth on saccharose agar, but they all produce the typical blue-green aerial mycelium on some medium, especially dextrose agar. Very characteristic is also the arrangement of long, fine, regular spirals as side branches of very long, only slightly branching aerial hyphae.

Genus Actinomyces, Group B.

The three strains of this group, whose general morphology has been described above, differ from each other in so many cultural characters that they cannot unreservedly be united into a single species, but on the other hand it would not seem advisable to erect three distinct species out of them on the basis of the study of so few strains.

Strain 6VI. Hab.—Soil 6. *Morphology.*—Vegetative hyphae on casein agar long, branching, of variable thickness, from 0·8–1·0 up to 2·5–3·0 μ. On nutrient agar and potato, transitions from long filaments to short rods (Pl. xix, fig. 12) and coccoid forms (Pl. xix, fig. 10), 1·0–1·2 × 1·2–1·5 μ. In dextrose-NH_4Cl-solution big globular forms, 2·5–3·0 μ (Pl. xix, fig. 9). Aerial hyphae on casein agar or potato extract agar, long, straight, branching, no spirals, about 1 μ thick. Spores short oval to nearly spherical, 1·0–1·2 μ (Pl. xix, fig. 6). *Cultural characters.*—

Saccharose agar: Good growth. Vegetative mycelium superficially spreading, raised, much wrinkled (lichnoid), cream-coloured, first of a loose consistence, later more firm and cartilaginous. Aerial mycelium develops slowly, as the growth becomes hard, of a dull-white colour. Dextrose agar: Good growth, rather similar to previous; aerial mycelium more abundant, cottony, pale yellowish-grey. Nutrient agar: Good growth. Soft, cream-coloured smear without aerial mycelium, first smooth, later slightly wrinkled, of entirely bacterium-like appearance. Potato: Fair growth. Flat, smooth, dirty cream-coloured, glistening smear, of a viscous and gum-like consistence. No aerial growth. Dextrose broth: Soft cream-coloured growth along edges of surface. Broth turbid when shaken.

Strain 6S. Hab.—Soil 6. *Morphology.*—Vegetative mycelium on casein agar similar to 6VI. On nutrient agar the hyphae mostly remain undivided, but in the condensation water of this medium, as well as in dextrose broth, potato, and dextrose asparagin solution, they divide into short rods (Pl. xix, fig. 8). Aerial hyphae on dextrose agar and casein agar long, tangled, without spirals, 0·8–1·0 μ thick. Spores long oval to barrel-shaped, 0·8–1·0 × 1·2–1·6 μ. *Cultural characters.*—Saccharose agar: Scant growth. Vegetative mycelium thin, deeply spreading, colourless. Aerial mycelium thin, tufted, zonate, first white, later pale pink. Dextrose agar: Vegetative mycelium raised, smooth, glistening, first cream-coloured, later ochre-yellow, surface first somewhat soft, later, when aerial mycelium develops, becoming hard. Aerial mycelium abundant, cottony, dull rose. Nutrient agar: Good growth. Vegetative mycelium raised, smooth, hard, yellowish-grey. No aerial mycelium. Pale yellowish-brown pigment. Potato: Abundant growth. Vegetative mycelium spreading, very much wrinkled, first cream-coloured, later dirty red-brown, of a soft consistence. No aerial mycelium. Yellowish-brown pigment.

Strain A III. Hab.—Soil A. *Morphology.*—Generally much like previous. On nutrient agar the hyphae divide after a few days into rather regular, oval to nearly spherical elements, 1·0–1·2 × 1·2–1·4 μ (Pl. xix, fig. 13), almost exactly reproducing Ørskov's (1923) figure of *Act. Affanassiew. Cultural characters.*—Saccharose agar: Fair growth. Vegetative mycelium superficial, wrinkled, glossy, first colourless, later faint brownish, firm, with a somewhat loose surface. Aerial mycelium well developed, smooth, pale ash-grey. Dextrose agar: Fair growth. Vegetative mycelium flat, pale yellowish-grey, with tufts of pale-grey aerial mycelium. Nutrient agar: Good growth. Vegetative mycelium superficial, raised, much wrinkled, cream-coloured, of a very soft consistence, entirely bacterium-like. Potato: Fair growth. Vegetative mycelium spreading, wrinkled, cream-coloured, first very soft, later becoming hard, when aerial mycelium develops. Aerial mycelium starts on tip of growth, spreads gradually downwards, pale-grey. Dextrose broth: Flaky cream-coloured sediment; broth faintly turbid.

Genus Proactinomyces.

Proactinomyces flavescens, n. sp.

Three strains. *Hab.*—Soils 129, 163, 176. This is one of the species of *Proact nomyces*, which show the closest resemblance to *Actinomyces*. Apparently it is a representative of that group of comparatively rare strains, which Lieske (1921) describes as "mittellang".

Morphology.—The vegetative mycelium varies much according to the medium. On media where a firm growth is produced it appears as long, branched, non-septate hyphae, 0·4–0·6 μ thick. In other media, e.g. nutrient agar and potato,

septation occurs, and the mycelium appears in preparations as fragments of very variable size, partly resembling highly branched mycobacteria. In several cases—for instance, on nutrient agar at 28–30° C., in 5–6 weeks old cultures in dextrose broth, and in dextrose-NH_4Cl-solution—the short elements assume swollen, fusiform to lemon-shaped forms (cf. Ørskov's (1923) description of *Act. corneae*). The aerial mycelium consists of fairly long hyphae of the same thickness as the vegetative hyphae, not very much branched, without spirals, often clinging together in wisps. A differentiation into spores is never visible by direct microscopical examination. Neither is this the case in stained preparations; here the aerial hyphae break up into fragments of quite variable length, from 1·2–1·5 up to 10–12 μ, showing an irregular, granulated staining. From this picture there is not a very wide step to that of *Act. albus*, as described above.

Cultural characters.—Saccharose agar: Good growth. Vegetative mycelium superficially spreading, much raised and wrinkled, cracking, white to cream-coloured, of a dry, but loose and crumbly consistence. Aerial mycelium scant, thin, white. Faint yellow soluble pigment after 2–3 weeks. Dextrose agar: Good growth. Vegetative mycelium superficial, wrinkled, honey-yellow to deep olive-yellow, of a hard and cartilaginous consistence. Aerial mycelium thin, smooth, white. Yellow soluble pigment. Nutrient agar: Good growth. Vegetative mycelium raised and much wrinkled, first dirty cream-coloured, later dark yellowish-grey, of a soft, moist, curd-like consistence. No aerial mycelium. No pigment. Potato: Good to excellent growth. Vegetative mycelium much raised and wrinkled, first cream-coloured, later yellowish-brown, soft and smeary. No aerial mycelium. No pigment. Dextrose broth: Rather scant growth. Granulated, yellowish sediment; no surface growth. Broth clear. No pigment. No acidity.

Biochemical features.—Saccharose is inverted. Starch is hydrolyzed. Cellulose is not decomposed. Gelatin is liquefied slowly. Nitrate is reduced slightly or not at all with various sources of energy. Milk is coagulated and slowly redissolved with acid reaction. Final reaction in dextrose-NH_4Cl-solution, pH 3·6–3·9. No growth under anaerobic conditions.

Proactinomyces paraffinae, n. sp.

Five strains. *Hab.*—Soils 163 and A. It appears somewhat problematic, in which genus this species-group should really be placed. Its mode of "spore" formation in the vegetative mycelium is quite like that in the aerial mycelium of *Actinomyces*, Group A. However, since it lacks the spore formation in the aerial mycelium and conforms with *Proactinomyces* in several other respects (acid-fastness, no diastatic action, no liquefaction of gelatin) it would seem more reasonable to include it in this latter genus.

Morphology.—In agar media (e.g., dextrose agar) the organism forms initially an extensive mycelium of long, richly branching hyphae, 0·4–0·5 μ thick. After 5–6 days at room temperature, numerous end branches swell to about double thickness, become more refractive, exhibit fine incisions along their external contours, and divide into oval, spore-like elements, 0·8–1·0 × 1·2–1·5 μ. This process of division starts at the tips of the swollen branches and proceeds basipetally (Pl. xx, fig. 15), until most of the hyphae appear divided. Primary septa are not seen in the hyphae even with the best optical equipment. A similar process of division takes place in liquid media, where also the filaments often fall into fragments of variable length. The spore-like elements, but not the undivided filaments, are markedly

acid-fast (Pl. xx, fig. 14). The aerial mycelium consists of rather short, straight, not very much branched hyphae, 0·4–0·6 μ thick, which never show any differentiation into spores.

Cultural characters.—Saccharose agar: Very scant growth. Thin colourless veil, sometimes with a trace of white aerial mycelium. Dextrose agar: Fair growth. Vegetative mycelium flat, growing into medium, pale ochre-yellow to orange, with raised outgrowths on the surface, of a crumbly consistence. Scant white aerial mycelium. Nutrient agar: Slow, but good growth. Vegetative mycelium superficial, somewhat raised, ochre-yellow, hard, but with a loose, smeary surface. Aerial mycelium scant, small white tufts. No pigment. Potato: Fair growth. Vegetative mycelium granulated, first pale-yellow, later deep ochre-yellow to orange. Scant white aerial mycelium. No pigment. Liquid media (milk, broth, synthetic solutions): Small round granules of various yellow to orange colours, firm, but can be crushed into a homogeneous smear. In quite old broth cultures a thick, hard, orange to brownish surface pellicle is formed.

Biochemical features.—Saccharose is not inverted. Starch is not hydrolyzed. Cellulose is not decomposed. Nitrate is not reduced. Gelatin is not liquefied. Milk is not coagulated or digested. Final reaction in dextrose-NH_4Cl-solution, pH 4·4–4·6. All strains show a marked power of utilizing paraffin wax as source of energy.

Proactinomyces polychromogenes (Vallée).

Syn. *Streptothrix polychromogenes* Vallée (1903).

This organism, which has previously been described in detail (Jensen, 1931*a*) under the name *Act. polychromogenes*, belongs quite evidently to the genus *Proactinomyces* (cf. Ørskov, 1923).

Proactinomyces actinomorphus (Gray and Thornton).

Syn. *Mycobacterium actinomorphum* Gray and Thornton (1928); *Actinomyces actinomorphus* (Gray and Thornton), Bergey (1930).

Four strains. *Hab.*—Soils A I, A II, 6, 163.

Morphology.—The organism varies considerably with the medium. In media permitting a good growth (nutrient or dextrose-asparagin-agar) there is, after 20–24 hours at 25–30° C., a formation of extensive mycelia of long, curled and richly branching hyphae, penetrating into the medium to a marked extent. Ordinary stained smear or impression preparates show only bacteria-like elements —curved and branched rods, 0·4–0·6 μ thick, of variable length (Pl. xx, fig. 20), gram-positive and non-acid-fast. Already, after two days, the mycelia divide into segments, often in angular arrangement, generally 3–6 μ long; coccoid forms are not seen. On media which allow only a poor growth (saccharose or glycerin nitrate agar or solution, water agar, etc.) the mycelial stage persists for a much longer time, and the organism appears microscopically as a typical actinomyces (Pl. xx, fig. 21–22); also here rod-shaped elements are formed in old cultures on the surface of agar media. In these meagre media, occasionally also on dextrose-agar, there is an abundant production of aerial hyphae; these are fairly straight and little branched, of the same thickness as the substratum hyphae (0·4–0·6 μ), and appear in stained preparations as rod-shaped fragments of varying length, not showing any differentiation into spores. The aerial mycelium tends to disappear after prolonged cultivation, especially in transfers from old cultures.

Cultural characters.—Saccharose agar: Fair growth. Substratum mycelium flat, thin, colourless, spreading deeply into the agar. Aerial mycelium abundant, smooth, snow-white, resembling chalk-powder. The surface growth becomes gradually wrinkled and soft. In Strain 163 the growth is very abundant, forming a thick, smooth, moist, cream-coloured smear of a soft, pasty consistence. No soluble pigment. Dextrose agar: Fair to good growth. Substratum mycelium first raised and wrinkled, white, later smooth, cream-coloured, of a very soft consistence. Smooth, snow-white aerial mycelium is formed early, but tends to disappear after 10–12 days. Nutrient agar: Good to excellent growth. Substratum mycelium forms a raised, smooth or wrinkled, soft, cream-coloured smear. No aerial mycelium. No pigment. Potato: Fair to excellent growth, forming a smooth, soft, spreading, yellowish-grey smear. No aerial mycelium or pigment. Dextrose broth: Good growth. Thick, white, soft sediment, later thin, white, fragile surface pellicle. Broth becomes turbid. No acidity. Gelatin: Filiform, cream-coloured growth along the stab. Slow, saccate liquefaction; liquefied gelatin clear, viscid, without pigment.

One of the strains of *Myc. actinomorphum* isolated by Gray and Thornton was found to agree with the present group in every respect except for failure to produce aerial mycelium on saccharose-nitrate-agar. This is perhaps not surprising in view of the much longer time it had been subjected to artificial cultivation. On dextrose-asparagine-agar some aerial mycelium was formed.

PROACTINOMYCES AGRESTIS (Gray and Thornton).

Syn. *Mycobacterium agreste* Gray and Thornton (1928); *Actinomyces agreste* (Gray and Thornton), Bergey (1930).

Five Strains. *Hab.*—Soils U, G, A I, 129, A III.

Morphology.—Cells sown on surfaces of agar grow out after 18–24 hours into small, but definite mycelia of an extent of up to 40–50 μ (Pl. xx, fig. 17). Some branches show a tendency to grow down into the agar. Septa are visible already at this stage, in the living state as well as in impression preparations (Pl. xx, fig. 16). Smear-preparations show only long branched rods, often in V- or Y-arrangement, 4–12 × 0·8–1·2 μ. The cells are gram-positive, non-acid-fast. The initial mycelia break up into long branching rods (Pl. xx, fig. 18), the ends of which sometimes show a tendency to bend and grow past each other ("slipping" growth). After 2–3 days at room temperature most of the cells in the interior of the colonies have divided into short rods and cocci, 1·2–3·0 × 0·8–1·2 μ. Round the edge of the colony one sees a number of rhizoid projections of long branched cells which remain undivided for a longer period and give a very characteristic appearance to the colonies (cf. Gray and Thornton, 1928). After 4–5 days these cells, too, have mostly divided into short rods and cocci, except the cells at the extreme tips of the projections, which still remain rod-shaped (Pl. xx, fig. 19). At higher temperatures (30–32° C.) the cells are often longer, 3–5 × 0·8–1·2 μ. In old cultures almost only the coccoid forms are seen.

Cultural characters.—Saccharose agar: Fair growth, smooth, convex, shining surface, butyrous consistence, edge entire, first cream-coloured, later pinkish to pale-greyish orange. Dextrose agar: Good growth, narrow, raised, smooth surface, finely myceloid edges, soft consistence, first white, later cream-coloured, finally pale-pink. Nutrient agar: Good growth, similar to previous, greyish-orange colour. Potato: Excellent growth, raised, restricted, finely rugose surface, dull yellowish-grey, soft. Dextrose broth: Good growth, first (two days) uniform turbidity,

later abundant cream-coloured sediment and thick fragile surface scum; no acidity. Gelatin: Filiform, white growth, with fine thread-like projections along line of stab; no liquefaction.—Distinct soluble pigments are not formed.

Dissociation.—The above description refers to the appearance of cultures as isolated from the soil. All strains showed, in platings from 2–5 months old broth cultures, a dissociation into two types of colonies: a "soft" type corresponding to the original, and a "hard" type, which produces a dry, wrinkled, firmly adherent growth in solid media, and in broth a tough surface pellicle, without any turbidity. Morphologically the two types are indistinguishable, except for the fact that the "hard" shows a rudimentary formation of aerial hyphae after 1–2 days on dextrose agar—short, straight, simple filaments, which soon disappear again. In the case of two strains (U and 129) these findings were confirmed with single-cell cultures obtained by the method of Ørskov (1922). The "hard" types have not so far reverted to the original. The whole phenomenon is apparently an analogy to the production of "plane" and "perrugose" varieties in the saprophytic mycobacteria (Haag, 1927).

The strains of this species studied by Gray and Thornton (1928) were all capable of attacking phenol and/or cresol, as a consequence of the selective method by which the cultures had been obtained. Of the present strains only two (G and U) were capable of attacking phenol; apparently this character is as variable as several other biochemical properties of this group, as shown by Gray and Thornton.

Proactinomyces minimus, n. sp.

One strain. *Hab.*—Soil from a flower pot.

Morphology.—Smear preparations from 3–4 days old agar cultures show 0·4–0·6 μ thick rods, bent and irregular, some branching or in V-position, of very variable length, from almost coccoid up to 8–10 μ long. Already after 10–12 days there are only few short rods (0·5–0·7 × 1·5–3·0 μ) left, and in older cultures (1–2 months) one sees only small cocci, 0·5–0·7 μ, mostly adhering into short chains or small clumps. The cells are gram-positive, but stain rather badly with ordinary dyes. The organism is acid-fast to a certain extent; in four-days-old cultures most cells are decolourized by the acid, although some retain the stain well; the small coccoid forms show good acid-fastness after six weeks. Direct observation on agar blocks shows a mode of development very similar to that of *Proact. agrestis*, from which the present organism differs mainly in the much smaller size of its elements, its acid-fastness, and its slower growth. There is here the same formation, after 2–3 days, of small mycelia, dividing into rods and finally cocci, and the same formation of burr-like colonies with rhizoid projections, in the tips of which the cells remain undivided for a longer time than those in the interior of the colony. Aerial growth is never observed.

Cultural characters.—The growth is most characteristic at room temperature. On potato and the agar media listed below, it is in general very slow, but ends with becoming quite abundant after 6–8 weeks; the consistence is that of a firm, crumbly paste. It is first colourless, later assuming a beautiful pink colour, most nearly corresponding to Flesh Pink (dextrose and nutrient agar) or Coral Pink (saccharose agar and potato), Rdg.* XIII. 5′ 00-R. f-d. Saccharose-nitrate-agar: Growth first thin and flat, later raised, restricted, with rugose surface and finely

* Ridgeway, Colour Standards and Nomenclature.

myceloid edges. Dextrose-asparagin-agar: Growth restricted, much raised and folded (walnut-kernel-like), with finely myceloid edges. Nutrient agar: Growth very much similar to the previous. Potato: Growth spreading, much raised, finely wrinkled (lichnoid). Dextrose-broth: Thin, broken, cream-coloured surface scum and sediment; broth clear. No acidity. Gelatin: Filiform, slightly granulated, cream-coloured growth along stab. No liquefaction.

Starch is not hydrolyzed. Cellulose is not attacked. Paraffin is utilized. No soluble pigments are formed.

Relative Abundance of Actinomyces *and* Proactinomyces *in the Soil.*

As previously mentioned, *Actinomyces* have been far more frequently found in the soil than *Proactinomyces*. This was also the case in the present work.

Table 1.
Comparative Physiology of *Actinomyces* and *Proactinomyces*.

Genus.	Organism	Diastatic Action.	Utilization of		Liquefaction of Gelatin	Growth on Paraffin	Final pH in Dextrose-NH_4Cl-Solution.	Brown Pigment in Protein Media.
			Xylan.	Cellulose.				
Actinomyces, Group A.	*Act. albus*	+	(+)	(+)	+	+	3·4–3·6	–
	„ *aureus*	+	+	–	+	–	3·4–3·6	+
	„ *bobili*	+	+	–	+	–	3·5–3·6	+
	„ *californicus*	+	–	–	+	–	4·5–4·7	–
	„ *exfoliatus*	+	(+)	–	+	–	4·5–4·7	–
	„ *flavus*	+	+	±	+	–	4·5–4·7	+
	„ *fulvissimus*	+	±	–	+	–	4·5–4·7	–
	„ *griseus*	+	(+)	–	+	–	4·9–5·0	–
	„ 218 W.	+	+	–	+	+	4·8–4·9	+
	„ *hygroscopicus* ..	+	±	±	+	–	3·7–3·8	–
	„ *microflavus*	+	–	–	+	–	5·1–5·2	–
	„ *parvus*	+	–	±	+	–	5·1–5·2	–
	, *reticuli*	+	–	+	+	+	4·1–4·2	+
	„ *roseochromogenus* ..	+	±	±	+	–	4·4–4·5	+
	„ *rutgersensis*	+	–	–	+	–	5·0–5·1	–
	„ *verne*	+	–	–	+	–	5·0–5·1	–
	„ *viridochromogenus* ..	+	+	(+)	+	–	5·0–5·1	+
Actinomyces, Group B.	*Act.* 6 VI	+	(+)	–	+	–	4·5–4·6	–
	„ 6 S	+	+	+	+	+	4·5–4·6	–
	„ A III	+	–	–	+	–	4·1–4·2	–
Proactinomyces.	*Proact. flavescens*	+	–	–	+	–	3·6–3·9	–
	„ *paraffinae* ..	–	(+)	–	–	+	4·4–4·6	–
	„ *polychromogenes* ..	–	–	–	–	+	4·4–4·6	–
	„ *actinomorphus* ..	+	–	–	+	+	4·9–5·1	–
	„ *agrestis*	–	–	–	–	+	4·4–4·6	–
	„ *minimus*	–	–	–	–	+	4·8–4·9	–

± indicates that the character is positive in some strains, negative in others.
(+) indicates that the character is present to a slight degree only.

While *Actinomyces*-colonies often account for 30–50% or more of the total number of colonies on plates of casein agar, it was rather exceptional to find a colony of *Proactinomyces*; it was necessary to make a special search for them in order to obtain the number of forms described above. Certain differences in the comparative physiology of the two genera might seem to offer an explanation for the relative preponderance of *Actinomyces*. As seen from Table 1, and as mentioned previously, all *Actinomyces* exert proteolytic activities (liquefy gelatin) and hydrolyze starch, many of them are capable of utilizing pentosan (Na-xylanate in mineral nutrient solution) and several of decomposing cellulose; *Proactinomyces* are generally non-proteolytic and non-diastatic, do not as a rule attack xylan, are always incapable of decomposing cellulose, but typically capable of utilizing paraffin.* It would, therefore, seem likely that the *Actinomyces* are better able to live on plant residues in the soil than the *Proactinomyces*. The table further shows that the resistance to acidity, which is quite characteristic for the strains within each single species-group (Jensen, 1930*b*), does not allow any distinction between *Actinomyces* and *Proactinomyces*.

A soil from a flower bed (heavy loam, rich in organic matter) gave, by plating in a dilution of 1:200,000 on 5 parallel plates of casein agar, 73 colonies of actinomycetes; 2 of these proved to be *Proactinomyces*; 1% of paraffin wax was added, and the moist soil was incubated for 1 month at room temperature, after which time a plating in a dilution of 1:500,000 on 5 parallel plates gave 303 colonies of actinomycetes, 30 of which proved to be *Proactinomyces* (*polychromogenes*, *agrestis*, and *paraffinae*). There is thus here a considerable increase, both absolutely and relatively, in the abundance of *Proactinomyces*, a result which indicates that these organisms may under special circumstances become important agents in the decomposition processes in the soil.

Summary.

A study was carried out on the morphology and biology of a number of strains of actinomycetes from Australian soils. They proved to fall into two main groups (apart from the genus *Micromonospora*), broadly corresponding to a division previously suggested by Ørskov.

I. Organisms producing an aerial mycelium which differentiates into spore-like bodies. Two subgroups could be distinguished: one in which the vegetative mycelium remains undivided, and one in which it divides into a kind of "fragmentation spores". The latter subgroup is of rare occurrence, and it is not certain that the two subgroups are sharply distinguished. All organisms of this group liquefy gelatin and hydrolyze starch, and several of them are capable of decomposing xylan and cellulose. Those actinomycetes which produce a characteristic brown pigment in protein media belong to this group, which includes the large majority of soil actinomycetes. It is suggested to reserve the generic name *Actinomyces* for this group. Seventeen species, among which one is new (*Act. hygroscopicus*), are described.

II. Organisms producing an aerial mycelium (sometimes nearly or wholly absent) without any differentiation into spores. The vegetative mycelium divides

* In a previous paper (Jensen, 1931*a*) it was erroneously stated that *Proact. polychromogenes* does not attack paraffin; this is, however, the case when it is grown in mineral nutrient solution with flakes of paraffin and not, as was previously done, on agar with addition of paraffin.

generally into bacteria-like segments which multiply in the same manner as the mycobacteria and corynebacteria, to which the transition from this group is very gradual. It is suggested that the formation of an initial mycelium be used as a criterion for the distinction of this group from the genera *Mycobacterium* and *Corynebacterium*. Organisms of this group often do not liquefy gelatin or hydrolyze starch and are always incapable of decomposing cellulose, but generally are capable of utilizing paraffin; several of them are acid-fast. These organisms are of rare occurrence in the soil; their abundance here can be increased by addition of paraffin to the soil. Many pathogenic actinomycetes belong to this group. It is suggested to classify this group as a separate genus, *Proactinomyces*, n. gen., to be included in the family Proactinomycetaceae Lehmann and Neumann. Six species are described; among these three are new (*Proact. flavescens, paraffinae,* and *minimus*). The order Actinomycetales can then be divided into the families Proactinomycetaceae (*Corynebacterium, Mycobacterium* and *Proactinomyces*) and Actinomycetaceae (*Actinomyces* and *Micromonospora*).

The sequence from *Actinomyces* to *Corynebacterium* and hence to "true" bacteria is very complete, and there is no reason to place the actinomycetes among the *Fungi imperfecti.*

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EXPLANATION OF PLATES XIX-XX.

Plate xix.

Fig. 1.—*Act. viridochromogenus.* Vegetative mycelium. Dextrose asparagin solution, 2 d. 28° C.—Fig. 2. *Act. parvus.* Vegetative mycelium. Nutrient agar, 16 d. 30° C.—Fig. 3. *Act.* 6 S. Vegetative mycelium. Condensation water of dextrose casein agar, 1 d. 30° C.—Fig. 4. *Act.* 218 Waksman. Aerial mycelium. Saccharose agar, 5 d. room tpt.—Fig. 5. *Act. albus.* Aerial mycelium. Dextrose agar, 8 d. room tpt.—Fig. 6. *Act.* 6 VI. Aerial mycelium. Potato extract agar, 4 d. room tpt.—Fig. 7. *Act.* 6 S. Aerial mycelium. Dextrose agar, 2 d. 30° C.—Fig. 8. Same. Vegetative mycelium. Condensation water of nutrient agar, 2 d. 30° C.—Fig. 9. *Act.* 6 VI. Vegetative mycelium. Dextrose-NH_4Cl solution, 20 d. 30° C.—Fig. 10. Same. Vegetative mycelium. Potato, 3 d. 30° C.—Fig. 11. *Act.* A III. Vegetative mycelium. Dextrose broth, 4 d. 28° C.—Fig. 12. *Act.* 6 VI. Vegetative mycelium. Nutrient agar, 5 d. room tpt.—Fig. 13. *Act.* A III. Vegetative mycelium. Edge of colony on nutrient agar, 5 d. 28° C. Living specimen.

Plate xx.

Fig. 14. *Proact. paraffinae.* Vegetative mycelium. Dextrose agar, 10 d. room tpt. Acid-fast-staining.—Fig. 15. Same. Dextrose agar, 8 d. room tpt. Living specimen.—Fig. 16. *Proact. agrestis.* Dextrose agar, 1 d. 30° C.—Fig. 17. Same. Dextrose agar, 20 h. room tpt. Living.—Fig. 18. Same. Dextrose agar, 2 d. room tpt. Living.—Fig. 19. Same. Dextrose agar, 5 d. room tpt. Living specimen.—Fig. 20. *Proact. actinomorphus.* strain A II. Nutrient agar, 1 d. 30° C.—Fig. 21. Same. Saccharose-nitrate solution, 7 d. 30° C.—Fig. 22. *Proact. actinomorphus,* strain Rothamsted. Condensation water of saccharose agar, 4 d. room tpt.

All specimens are stained with dilute carbol fuchsin, unless otherwise stated. In Figs. 13, 17, 18 and 19 the magnification is × 350, in all other cases × 750.